Report for 2005MT51B: STUDENT FELLOWSHIP: Antibiotic resistance in ground-and surface-water microbes in the Missoula area

Publications

- There are no reported publications resulting from this project.

Report Follows

Research Summary, Water Center Fellowship Research Work
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Spotted knapweed is a highly invasive weed species in Montana, where invasion often results in massive disruption of stream-side flora, causing increased sediment runoff into waterways, and the associated declines in certain aquatic plant and animal species. Following recent research which showed that Spotted knapweed (Centaurea maculosa) is responsible for the secretion of two enantiomeric forms of the flavenoid chemical *catechin*, and reports that catechin may exhibit antimicrobial properties, we decided to investigate the specific effects of catechin exposure on common soil bacteria. Because soil bacterial communities are critical to the survival of many plant species, we hypothesized that catechin may be responsible for the major disruption of intact soil microbial communities on which native flora rely for survival. Our aim was to determine whether catechin exposure was inhibitory to specific soil bacteria.

Over the course of the past year, we have performed a number of experiments in the laboratory, including the following:

(1) ~ 500 microbial strains isolated from soil samples from knapweed-present and knapweed-absent sites were tested for resistance to catechin at environmentally significant concentrations. Percentages of overall resistance to catechin were calculated using Most Probable Number analyses, plate counts and UV/vis spectroscopy. ~ 20 strains of special interest were then isolated for future experimentation and identified by DNA sequencing.

(2) Isolated strains were tested for growth success over time, under different conditions including no catechin; high and low steady-state catechin levels; decreasing catechin levels; periodically re-applied catechin exposure; and catechin exposure followed by complete removal of catechin from the system.

(3) Isolated strains were tested for growth success over time when exposed to catechin in the presence of varying carbon source substrates. API carbon usage and enzyme tests were performed on several isolates of interest.

(4) Isolates capable of sporulation were tested for spore-formation success and recovery success using spectrophotometry when sporulation and recovery were induced in the presence of catechin.

(5) The abiotic stability of catechin over time in liquid media with varying chemical properties was examined using HPLC analysis, including factors such as varying pH; addition of specific metals and chelators; and addition of specific organic acids.

Results of the experiments showed a varied and somewhat complex relationship between soil bacteria and catechin exposure. At low concentrations (500 ppm) catechin was minimally inhibitory but at higher concentrations (2000-3000 ppm), catechin was highly inhibitory, inhibiting growth in ~ 60 to 80% of bacterial species tested.

Catechin appears to be a microbistatic compound, rather than an antimicrobial: following removal of catechin from an experimental system, inhibited microbes were able without exception to resume normal growth. In microbial systems where catechin is added initially but not reapplied, catechin concentration usually drops steadily, with catechin apparently converting to another compound, and is followed by very slow recovery of microbial growth…though whether this conversion of catechin is due to biological activity or an abiotic chemical reaction is uncertain.

Utilizing different carbon sources available for microbial utilization did not noticeably change resistance/susceptibility to catechin by the isolated organisms, with the exception of the organic acid *pyruvate*. None of the organisms tested were inhibited by catechin when grown in the presence of pyruvate as the sole carbon source. The biochemical basis of this discovery is still under investigation.

Catechin's effects on sporulation were not uniform. For some organisms, sporulation and recovery from spores were both unaffected. For other organisms, sporulation was inhibited, but not recovery from sporulation, and in other cases only recovery from sporulation was inhibited. The biochemical basis of these results are also still under investigation, but calcium binding by catechin may be a factor.

Catechin proved to be highly stable over time at low pH (~ 4) and highly unstable at high pH (~ 9). Addition of metals to abiotic catechin media produced varying results, with calcium addition causing the highest catechin stability and copper addition resulting in the greatest level of catechin instability. The effect of organic acids on catechin stability did not appear significant.

In conclusion, catechin *does* appear to be highly inhibitory to many soil microbial genera. Some genera, however, appear naturally resistant, including *pseudomonas* and *rhodococcus* species. Catechin is not highly inhibitory under all conditions, and the factors which govern its inhibitory properties are many and varied. Because we now have a much better understanding of microbial inhibition by catechin in the lab environment, the next step is to proceed in *in situ* analysis of microbial communities, using potting experiments and microbial community DNA extraction techniques. The combination of controlled *in vitro* experiments and observation of catechin-microbial interactions in real *in situ* conditions should allow us to make accurate conclusions about the validity of the hypothesis that knapweed invades by means of disruption of soil microbial communities.